Bioactive Compounds in Bael Fruit Pulp Waste: Ultrasound-Assisted Extraction, Characterization, Modeling, and Optimization Approaches

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Abstract: Bael fruit is an abundant source of bioactive compounds that have importance in the food and pharmaceutical industries. The process of extraction of the bael fruit juice produced a higher percentage of bael fruit pulp waste (BFPW) (37.33 %), which was recycled for the extraction of bioactive compounds. Thus the bioactive compounds like polyphenols, flavonoids, and carotenoids were extracted from BFPW by using the ultrasound-assisted extraction (UAE) technique. The modeling and optimization of the extraction process were carried out by using the experimental design of response surface methodology (RSM). The ethanol concentration of 51.22 %, ultrasound amplitude of 51.45 %, and ultrasound treatment time of 6.11 minutes were obtained to be an RSM optimized values of extraction process variables. The lower values of root mean squared error (RMSE) and mean absolute error (MAE) and higher values of coefficient of determination (R^2) indicated admissibility and acceptability of RSM. This extraction process of bioactive compounds has the potential to implement it on an industrial scale for the formulation of food additives and medicines.

Keywords: Bael fruit pulp waste; bioactive compounds; ultrasound-assisted extraction; response surface methodology (RSM).

1. Introduction

Bael (*Aegle marmelos* C.) is one of the most important indigenous medicinal fruit which grows in tropical and subtropical regions of Asia. Various parts of the bael tree, i.e., fruits, leaves, stem, roots, and bark, are found to be medicinally useful [1-4]. Among these parts, underutilized bael fruit possesses more nutritive values and can be processed to various products such as jam, jelly, juice, candy, squash, powder, capsules, toffee, wine, etc. The bael fruit is found to contain bioactive compounds, namely pectin, tannins, flavonoids, alkaloids, carotenoids, phenolic acid, terpenoids, and coumarins [5-7]. The presence of bioactive compounds imparts many health benefits in edible bael fruit and its products with antidiabetic activity, anticancer activity, antioxidant activity, antihistaminic activity, antiamoebic activity, antibacterial activity, antiulcer activity and, anti-inflammatory activity [8-10].

The processing of fruits and vegetables produces industrial solid waste such as rind, skin, seed, core, pod, pomace, peels, etc. which are the potential source of bioactive and nutraceutical compounds. This solid waste has grabbed the attention of researches for the valorization or extraction of the essential compounds [11-13]. The recycling or reuse of

industrial food solid waste for the extraction of essential compounds is found to be useful as it helps to eradicate environmental pollution [14,15]. During the processing of bael fruit products, many waste material, i.e., hard rind or peel, fibers, seeds, etc. are obtained. They also contain bioactive and pharmaceutical compounds [16]. The delicious 'sherbat' or bael fruit juice has been reported to possess tonic, laxative, restorative, and digestive properties and can be used for preparing jam, jelly, wine, and squash [17-20]. Therefore, Singh *et al*. (2013) have commercialized the bael fruit into nutritious juice by using enzymatic hydrolysis of bael fruit pulp. The cake of fiber or retentate has been obtained in muslin cloth after the mechanical expression of juice. This cake of fibers or retentate is considered a byproduct of solid waste of the bael fruit juice extraction process and termed as a bael fruit pulp waste (BFPW) [7]. Various bioactive and pharmaceutical compounds can be recovered from BFPW as bael fruit pulp is an abundant source of many bioactive and pharmaceutical compounds such as β-carotene, marmelosin, luvangetin, aurapten, psoralen, marmelide, riboflavin, flavonols, flavonoid glycosides, leucoanthocyanins, umbelliferone, and anthocyanins [6,21]. These compounds cannot be available easily in any other fruits and have particular importance in the pharmacy for preparing the food additives and medicines. The extracted bioactive and pharmaceutical compounds also act as medicinal additives, which can be incorporated in some medicines [7,22,23]. So the BFPW can potentially be used for the extraction of bioactive and pharmaceutical compounds.

Several methods of bioactive and pharmaceutical extraction have been implemented in medicinal and food industries from plant material or food solid waste. These methods include solid-solvent extraction, supercritical fluid extraction, accelerated solvent extraction, soxhlet extraction, enzyme-assisted extraction, microwave-assisted extraction, ultrasonic-assisted, and ultrasound-assisted extraction (UAE) [24-32]. The conventional methods of extraction, such as heating, boiling of refluxing hydrolyze, degrade, ionize, and oxidize the targeted bioactive compounds leading to lower extraction efficiency [25]. The ultrasound-assisted extraction of bioactive compounds can be deemed as a novel, environment-friendly, low-cost technique with higher extraction efficiency [31,33,34]. The ultrasound-assisted extraction is a non-thermal technique that allows the more retention of bioactive compounds during extraction. The ultrasound cavitation produces shear stress in the cell wall. It results in the mechanical breakdown of cell walls, which allows more diffusion rate or material transfer across the cell wall [35,28,36,37]. The ultrasound-assisted extraction (UAE) technique has been reported in many plants, food, and waste materials for the extraction of bioactive or essential compounds. The previous works on the UAE of essential compounds have been shown in Table 1.

The bioactive compounds present in fruits mainly comprise of polyphenols, flavonoids, and carotenoids [53-55,45]. The polyphenols, which are categorized as phenolic acids, stilbenes, flavonoids, and lignans, exhibit the antioxidant activity and can be useful for heart diseases. Flavonoids include flavanols, anthocyanidins, isoflavones, flavones, flavanones, etc., which can prevent oxidation, coronary heart disease, and cancer of membrane lipids [56,57]. The lutin, β-carotene, zeaxanthin, α- carotene, β-cryptoxanthin, etc. are included in carotenoid, which decreases the risk of cancer, chronic diseases, and other degenerative [58].

This paper includes the process of ultrasound-assisted extraction (UAE) of bioactive compounds from the bael fruit pulp waste (BFPW). The article also covers the modeling and optimization of the extraction process by using response surface methodology (RSM) for its Commercialization on an industrial scale.

Table 1. Extraction of bioactive compounds by using ultrasound treatment.

Materials	Analytes	References
Pomegranate wastes	Carotenoids	[38]
Peaches and Pumpkins	Phenolic compounds	[39]
Haskap berries (*Lonicera caerulea* L.)	Anthocyanins	[40]
Spinach extract	Polyphenols	[41]
Grapefruit (*Citrus paradisi* L.) solid wastes	Flavonoids	[42]
Carrot (*Daucus carota* Linn)	Carotenoids	[43]
Marjoram (*Origanum majorana* L.)	Antioxidant compounds	[44]
Inula helenium	Flavonoids	[45]
Mangifera pajang Kosterm. peels	Phenolic compounds	[46]
Prunella vulgaris L.	Flavonoids	[47]
Orange (*Citrus sinensis* L.) peel	Polyphenols (flavanone glycosides)	[31]
Du Zhong Ye (*Folium eucommiae*)	Flavonoids	[48]
Penggan (*C. reticulate*)	Total phenolic content	[49]
Satsuma mandarin (*C. unshiu Marc*)	Flavanone glycosides and phenolic acids	[50]
Pomegranate (*Punica granatum* L.) peel	Phenolic compounds	[51]
Almond extract	Phenolics, tannins, anthocyanin, and total flavonoids	[52]

2. Materials and Methods

2.1. Process for bael fruit juice extraction and its byproduct.

The Kagzi variety of Bael fruits were harvested from trees, which are located on the campus of the National Institute of Technology, Rourkela (Location: 22.2604° N, 84.8536° E). Fruits were stored in a deep freezer at -20 ± 2℃. The ripen fruits (orange-yellowish color) were broken by using the hammer, and pulp was scooped along with seeds and fibers. The scooped pulp was passed through an IC30 sieve to separate the seeds and large fibers from the pulp. The water was added to the fined texture sieved pulp in the proportion of 2:1 (water: pulp) to obtain bael fruit juice easily. The bael fruit juice was extracted enzymatically by using pectinase enzyme (EC-232-885-6) (activity: 8000-12000 units/g, commercial name: Himedia, Mumbai, India) of 0.20 mg per 100 g of pulp at 45℃ for 6 hours and then passing it through muslin cloth with the application of mechanical pressure. The byproduct of the bael fruit juice extraction process, i.e., cake or retentate or bael fruit pulp waste (BFPW), remained in the muslin cloth after extraction of juice. The collected BFPW was used as a primary sample for the extraction of bioactive compounds. The mass balancing of bael fruit juice and its byproduct, i.e., BFPW, was done for the calculation of percentage waste of bael fruit pulp. The moisture content of sieved bael fruit pulp and BFPW was determined gravimetrically at 105 ± 2℃ for 24 hours [59].

2.2. Extraction and separation of bioactive compounds.

The homogeneous mixture of 50 mL of ethanol and 10 g of BFPW was allowed for ultrasound treatment. The solvent volume to sample ratio was kept constant throughout the experiment. A probe-type ultra-sonicator (Model: Q700 Sonicator, Qsonica Sonicator, USA) with a maximum power rating of 700 Watt and a frequency of 20 kHz was used for ultrasound treatment. The power of ultrasound was controlled by its amplitude, which ranges from 1 to 100%. A standard probe of ½" diameter was used to generate the ultrasounds with the pulse-on time of 10 seconds and a pulse-off time of 10 seconds. The probe was dipped 1.5 times the diameter of the glass beaker, which contains the sample. The temperature of BFPW was maintained below 45℃ during ultrasound treatment [60,61]. After ultrasound treatment, the sample was centrifuged at 12000 rpm for 5 minutes at 25℃ in decanter centrifuge (Model: Sigma 2-16KL, Sigma Laborzentrifugen GmbH, Germany) to separate the solids of BFPW

from the extract of bioactive compounds. The separation of ethanol from the extract was carried out in a rotary vacuum evaporator at 130 mbar pressure and 45℃ for 30 minutes with a speed of 150 rpm [31,47]. The ethanol-free extract of bioactive compounds was stored at -20 ± 2℃ for further analysis. The conventional solid-liquid extraction method of the bioactive compound was performed to compare with the UAE [42]. The process of extraction of bioactive compounds from BFPW is shown in Figure 1.

Figure 1. Process for ultrasound-assisted extraction of bioactive compounds.

2.3. Experimental design.

Three operational variables, i.e., ethanol concentration (X1), ultrasound amplitude (X2), and ultrasound treatment or process time (X3), were considered for the extraction of bioactive compounds. The lower (-1) and upper (+1) limits of operational variables were set by performing primary experiments. The coded values, i.e., -1 and +1, correspond to the lower and upper limits of actual values, respectively. The ethanol concentration was varied from 30 (-1) to 90 (+1)% (%v/v) by dilution with distilled water. The ultrasound amplitude was varied across the range from 40 (-1) to 80 (+1)%. The Ultrasound treatment time was set from 4 (-1) to 10 (+1) minutes. The . Box–Behnken design of response surface methodology (RSM) was carried out to get the total runs (experimental trials) of different combinations in Design-Expert software 11.0. Box–Behnken design of three variables gives 17 experimental trials.

2.4. Characterization of extracted bioactive compounds.

After the separation of ethanol from the extracts of bioactive compounds, the remaining extracts were made up to the volume of 50 mL with distilled water to equalize their volumes for the determination and comparison of extracted bioactive compounds. The characterization comprised the determination of total phenol content, total flavonoids, total carotenoids, and DPPH scavenging activity.

Total phenol content (TPC) was measured spectrophotometrically by using the Folin-Ciocalteu assay, which was described by Singleton and Rossi (1965) with slight modifications [62]. The Folin-Ciocalteu reagent was diluted 10-fold with distilled water. The extract of 125 μL was then added to 1.8 mL diluted Folin-Ciocalteu reagent. The prepared solution was

allowed to stand for 5 minutes. The solution of 15% sodium carbonate was then added to the prepared solution. The absorbance was measured at 765 nm after 90 minutes of incubation time at room temperature. The TPC was expressed in mg of gallic acid equivalents per mL of extract after comparing the absorbance with the standard graph of gallic acid with the equation: $y = 1.4584x + 0.0629$ ($R^2 = 0.9932$).

Total flavonoids (TF) were determined as per the procedure described by Woisky and Salatino (1998) with slight modification [63]. The extract of 0.5 mL was added to 0.5 mL of 2% $AlCl_3$ and 2.5 mL of 99 % methanol. After incubation for 15 min at room temperature, the absorbance was measured by UV-vis Spectrophotometer (Model: AU 2701, Systronics, India) at 420 nm. The TF was expressed in μm of rutin equivalents per mL of extract as rutin was used as the standard for obtaining the curve of the equation: $y = 1.4784x + 0.0471$ ($R^2 = 0.9882$).

Total carotenoid (TC) was determined according to the method of Hess *et al.* (1991) with some modifications [64]. The extract of 300 μL was added to 300 μL of water and 600 μL of ethanol to make an ethanolic solution. Then it was extracted to 600 μL of n-hexane. The absorbance was measured at 450 nm in UV-vis Spectrophotometer (Model: AU 2701, Systronics, India). The results were expressed in μg of β-carotene equivalents per mL of extract. The standard curve of β-carotene has shown the equation: $y = 2.0051x + 0.0693$ with R^2 of 0.9835.

The method of Liu *et al.* (2009) was implemented for the determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity with some modifications [65]. The DPPH solution was prepared daily with a concentration of 0.4 mmol/L of DPPH in 95% ethanol. The extract of bioactive compounds was diluted with distilled water at a dose of 1 mL of extract per 4 mL of water. The 2 mL diluted extract was added to 2 mL of DPPH solution and 2 mL of 95% ethanol each. Then the mixtures were shaken vigorously and allowed for 30 minutes of incubation time at ambient room temperature in a dark place. The absorbance was taken at 517 nm against the blank. The DPPH scavenging activity was calculated by the following equation.

$$\text{DPPH scavenging activity (\%)} = \left[1 - \frac{(A_i - A_j)}{A_c}\right] \times 100 \qquad (1)$$

where A_c= 2 mL of DPPH solution + 2 mL of 95% ethanol, A_i= 2 mL of diluted extract + 2 mL of DPPH solution and A_j= 2 mL of diluted extract + 2 mL of 95% ethanol.

The results of TPC (mg), TF (mg), and TC (μg) were determined per mL of the extract of bioactive compounds from their respective standard curve equations and finally represented per 100 mL of the extract of bioactive compounds. All analyses were performed in triplicates for every run, further taking the mean values along with the standard deviation.

2.5. RSM model and optimizations.

The responses, namely TPC (Y1), TF (Y2), TC (Y3), and DPPH scavenging activity (Y4), were taken into account in RSM as the main targeted parameters. The effect of variables such as ethanol concentration (X1), ultrasound amplitude (X2), and ultrasound treatment time (X3) on responses was performed and studied by developing different mathematical regression equations, which may contain linear, quadratic, and interaction terms. The second-order polynomial regression equation of RSM was used to express the responses as a function of variables, as shown in equation 2 [46,66].

$$Y = \beta_0 + \sum b_1 X_1 + \sum b_2 X_2 + \sum b_3 X_3 + \sum b_{12} X_1 X_2 + \sum b_{13} X_1 X_3 + \sum b_{23} X_2 X_3 + \sum b_1{}^2 X_1{}^2 + \sum b_2{}^2 X_2{}^2 + \sum b_3{}^2 X_3{}^2 \qquad (2)$$

where Y is predicted response, b_1, b_2, and b_3 are the linear coefficients, b_{12}, b_{13}, and b_{23} are the interaction coefficients, whereas b_{12}, b_{22}, and b_{32} are the quadratic coefficients. The RSM models or equations of responses were validated by using an analysis of variance (ANOVA) of Design-Expert software 11.0. Three-dimensional plots (response surfaces) were obtained by using RSM polynomial equations to study the effect of variables on responses [67].

All four responses, i.e., TPC, TF, TC, and DPPH scavenging activity, were assigned as maximum parameters to obtain the optimized values of variables for the extraction of bioactive compounds. The RSM optimization was carried out in the numeric optimization tool of Design-Expert software 11.0. The corresponding values of responses were determined from the optimized values of variables for RSM optimization [39].

2.6. Validation of the RSM model.

The coefficient of determination (R^2), mean absolute error (MAE), and root mean squared error (RMSE) was calculated for the validation of developed RSM models or equations. The formulae of R^2, MAE, and RMSE are given below [68,69].

$$R^2 = \left(\sum_{i=1}^{n}(Yi_{pre} - Yi_{obs})^2\right) / \sum_{i=1}^{n}(Yi_{pre} - \overline{Y})^2 \qquad (3)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n} |(Yi_{obs} - Yi_{pre})| \qquad (4)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Yi_{obs} - Yi_{pre})^2} \qquad (5)$$

where Yi_{obs} is an observed value of the response, Yi_{pre} is RSM predicted value of the response, $\overline{Y}$ is the average value of the observed responses, and n is the number of data points for a single response.

3. Results and Discussion

3.1. Mass balancing of the bael fruit juice extraction process.

The moisture content of sieved bael fruit pulp was found out to be 68.25±1.25% (wet basis). The mass balancing was done on the basis of weights. The sieved bael fruit pulp of 3 kg and 6 kg of water (1:2 ratio of pulp to water) were mixed thoroughly and then allowed for enzymatic extraction of juice. The clear bael juice of 7.74 kilograms (juice yield of 58%) was extracted from 9 kg homogeneous mixture of sieved pulp and water with handling losses of 0.14 kg. The bael fruit pulp waste (BFPW) was recovered from the muslin cloth after extraction of juice and weighed to be 1.12 kg. The percentage of waste obtained from sieved bael fruit pulp during the juice extraction process was determined to be 37.33 %. The presence of fibers contributes to the complex nature of bael fruit pulp, which produces a higher percentage of waste, i.e., BFPW, along with bioactive compounds [70]. The BFPW was found to have a moisture content of 62.45±1.22% on the wet basis, and this confirmed the higher water holding capacity of fibers (cellulose), which are present numerously in the bael fruit pulp [71].

3.2. Conventional extraction versus UAE.

The bioactive compounds were extracted by the conventional method of solid-liquid extraction from BFPW. The conventional solid-liquid extraction methods involved the addition of water to the BFPW, and heating of water added BFPW for some time. The water-added sample of BFPW was heated to 60°C for 30 minutes [42]. The conventional extraction of bioactive compounds has shown the values of TPC, TF, TC, and DPPH scavenging activity as 37.25±1.45 mg/100 mL, 15.78±1.04 mg/100 mL, 2.54±0.96 μg/100mL, and 41.47±1.27% respectively. These values were found to be effectively lower than the ultrasound-assisted extracted bioactive compounds. The superiority of UAE over conventional extraction has been reported in many food types of research [31,42,72,41,73,39]. The conventional solid-liquid extraction process gives less extraction efficiency of bioactive compounds as it comprehends higher temperature and extraction time, which degrade or denature the polyphenols, flavonoids, and carotenoids. Consequently, it reduces overall antioxidant activity or free radical scavenging activity [74-78,38]. In contrast, ultrasound-assisted treatment gives higher extraction efficiency of bioactive compounds, as it is a non-thermal processing technology that works on the principle of cavitation [47,72,79,80,73,45,44]. Many pharmaceutical industries, therefore, have been using ultrasound-assisted extraction technique by coupling with other non-thermal extraction techniques for obtaining the higher extraction efficiency of bioactive compounds.

3.3. RSM analysis.

The Box–Behnken design of response surface methodology (RSM) has developed the quadratic equations (models) for every response. The predicted values of responses, namely TPC, TF, TC, and DPPH scavenging activity, were determined by the RSM quadratic equation, which was in the form of equation 2. The observed values with their standard deviation and RSM predicted values of responses are given in Table 2. The developed models of quadratic equations have given the values of regression coefficients. The regression coefficients of linear, interaction, and quadratic terms with their significance are shown in Table 3. The significance of quadratic models, as well as lack of fit, were determined by ANOVA. F-values of 57.61, 39.55, 29.99, and 40.25 for TPC, TF, TC, and DPPH scavenging activity respectively have indicated that the developed models are significant ($p < 0.0001$).

The lack of fit for all models was found out to be insignificant as F-values of lack of fit were 2.45, 1.92, 0.5267, and 3.45 for TPC, TF, TC, and DPPH scavenging activity, respectively. The insignificancy of a lack of fit is desirable and acceptable for the model to fit [60,81]. The coefficient of determination (R^2) value should be higher than 0.80 for the best fit model [82]. The R^2 values of all developed models were determined to be more than 0.80, as mentioned in Table 3. This indicates that the developed RSM models are the best fit models. The difference of less than 0.20 agreed to the reasonable relation between adjusted and predicted R^2 for all models. The values of mean absolute error (MAE) and root mean squared error (RMSE) are shown in Table 3. These values were found out to be within the acceptable limits corresponding to their observed and predicted values of responses [83,18].

Table 2. Observed and RSM predicted values of TPC, TF, TC, and DPPH scavenging activity.

Runs	Ethanol concentration %	Ultrasound amplitude %	Ultrasound treatment time minutes	TPC mg/100mL		TF mg/100mL		TC µg/100mL		DPPH scavenging activity %	
				Obs	Pre	Obs	Pre	Obs	Pre	Obs	Pre
1	60	60	7	68.95±2.53	68.39	43.10±1.83	41.82	6.62±0.33	6.89	75.45±2.93	76.21
2	90	60	10	54.29±2.08	54.20	31.36±1.45	31.54	5.35±0.31	5.30	55.88±2.86	55.77
3	90	60	4	51.87±2.11	51.58	35.42±1.56	35.17	5.78±0.24	5.67	47.28±2.71	48.10
4	90	40	7	60.09±1.98	59.44	36.23±1.48	35.31	5.14±0.21	5.22	53.97±2.83	51.89
5	60	40	4	70.54±2.34	71.48	52.40±1.67	53.57	5.35±0.20	5.38	70.97±2.98	72.23
6	90	80	7	49.73±2.00	50.76	30.28±1.99	31.27	4.92±0.27	5.00	51.19±2.95	52.56
7	60	60	7	67.68±2.24	68.39	41.75±1.86	41.82	7.09±0.45	6.89	78.62±2.82	76.21
8	30	60	10	65.58±2.56	65.87	45.16±1.78	45.41	6.21±0.39	6.32	56.25±2.77	55.43
9	60	60	7	68.68±2.44	68.39	41.75±1.69	41.82	6.85±0.30	6.89	74.76±2.54	76.21
10	60	40	10	69.97±2.61	70.71	48.89±1.62	49.63	5.78±0.28	5.75	70.33±2.69	72.52
11	60	80	10	67.73±2.39	66.79	46.94±1.74	45.77	5.14±0.25	5.11	78.38±2.84	77.12
12	30	60	4	65.59±2.78	65.68	48.40±1.45	48.22	5.99±0.41	6.04	62.60±2.89	62.71
13	30	40	7	70.79±2.71	69.76	50.30±2.01	49.31	5.99±0.43	5.91	56.35±2.73	54.98
14	30	80	7	65.57±2.15	66.22	43.26±1.77	44.18	5.78±0.19	5.70	61.65±2.92	63.73
15	60	80	4	63.93±2.26	63.19	48.99±1.89	48.25	5.56±0.41	5.59	79.22±2.90	77.03
16	60	60	7	67.22±2.47	68.39	42.48±1.63	41.82	7.06±0.22	6.89	75.17±2.74	76.21
17	60	60	7	69.40±2.05	68.39	40.02±1.60	41.82	6.85±0.36	6.89	77.03±2.60	76.21

(Obs: observed; Pre: predicted)

Table 3. Regression coefficients of the RSM model.

Regression coefficients	TPC	TF	TC	DPPH scavenging activity
β_0	68.39	41.82	6.89	76.21
b_1	-6.44***	-6.73***	-0.35***	-3.57**
b_2	-3.05***	-2.29**	-0.11*	2.35*
b_3	0.71*	-1.61**	-0.03	0.10*
b_1b_2	-1.29*	0.27	0.00	-2.02
b_1b_3	0.61	-0.21	-0.16*	3.74**
b_2b_3	1.09	0.37	-0.21*	-0.05
b_1^2	-7.78***	-5.51***	-0.53***	-19.82***
b_2^2	0.93	3.71***	-0.91***	-0.60
b_3^2	-1.28*	3.78***	-0.53***	-0.88
R^2	0.99	0.98	0.97	0.98
Adjusted R^2	0.97	0.96	0.94	0.96
Predicted R^2	0.85	0.81	0.86	0.77
MAE	0.6600	0.7282	0.0870	1.30
RMSE	0.7398	0.8744	0.1094	1.47

(*Significant at $p < .1$; **Significant at $p < .05$; ***Significant at $p < .001$).

3.4. Effect of variables on the extraction of bioactive compounds.

The effect of operational variables, i.e., ethanol concentration, ultrasound amplitude, and ultrasound treatment time on the TPC, TF, TC, and DPPH scavenging activity, was studied by the responses surfaces (three-dimensional graphs) which were obtained from the equations of developed models. The operational variables showed a greater effect on the extraction process of bioactive compounds because all models were significant ($p > .05$) as per the results of ANOVA [84].

The total phenol content (TPC) ranged from 49.73 to 70.79 mg gallic acid equivalent per 100mL of extract for the extraction process. Figure 2 (i-a) shows the highest TPC at a lower amplitude and in the middle range of ethanol concentration. The ultrasound treatment time has shown a negligible effect on the extraction of polyphenols (Figure 2 (i-b and c)). The ethanol concentration and ultrasound amplitude had a negative significant linear impact on TPC; in contrast, the ultrasound treatment time had a positive significant linear effect on TPC. Higher amplitude may increase the cavitation, which can cause the degradation of polyphenols [39]. The ethanol enhances the diffusivity and solubility of polyphenols by lowering the dielectric constant of BFPW. The higher concentration of ethanol makes the diffusion very difficult by denaturing the protein cell wall [85]. Similar kinds of the effect of ethanol concentration on TPC were obtained by Hossain *et al*. (2012), Prasad *et al*. (2011), and Altemimi *et al*. (2015), which showed a similar trend of TPC extraction for ultrasound treatment.

The total flavonoids (TF) were found to be ranged from 30.28 to 52.48 mg rutin equivalent per 100mL of extract. Figure 2 (ii-a) represents the higher extraction of flavonoids in the middle range of ethanol concentration and at either lower or higher amplitude of ultrasound treatment. Similarly, in Figure 2 (ii-b and c), the extraction process has given the highest flavonoid content, either lower or a higher range of ultrasound treatment time. The ethanol concentration, ultrasound amplitude, and ultrasound treatment time have shown a negative significant linear effect on the extracted TF. The moderate concentration of ethanol for higher extraction of TF may indicate the medium polar components present in the BFPW, whereas middle ranges of ultrasound amplitude and treatment time may attain saturation condition for denaturation of flavonoids, which is not favorable for higher extraction of flavonoids [74]. The results of TF supports the findings of Garcia-Castello *et al*. (2015), Wang *et al*. (2012) and Altemimi *et al*. (2015).

The total carotenoids (TC) is the major targeted bioactive compounds for UAE. TC had an extraction range from 7.09 to 4.92 μg β-carotene equivalents per 100 mL of extract. The middle ranges of ethanol concentration, ultrasound amplitude, and ultrasound treatment time have given the highest content of extracted carotenoids, as shown in Figure 3 (iii). The negative significant linear effect has been shown by ethanol concentration and ultrasound amplitude on the TC, whereas ultrasound treatment time had a linear non-significant impact on TC. The results of ethanol concentration on TC are in agreement with the results of Goula *et al.* (2017).
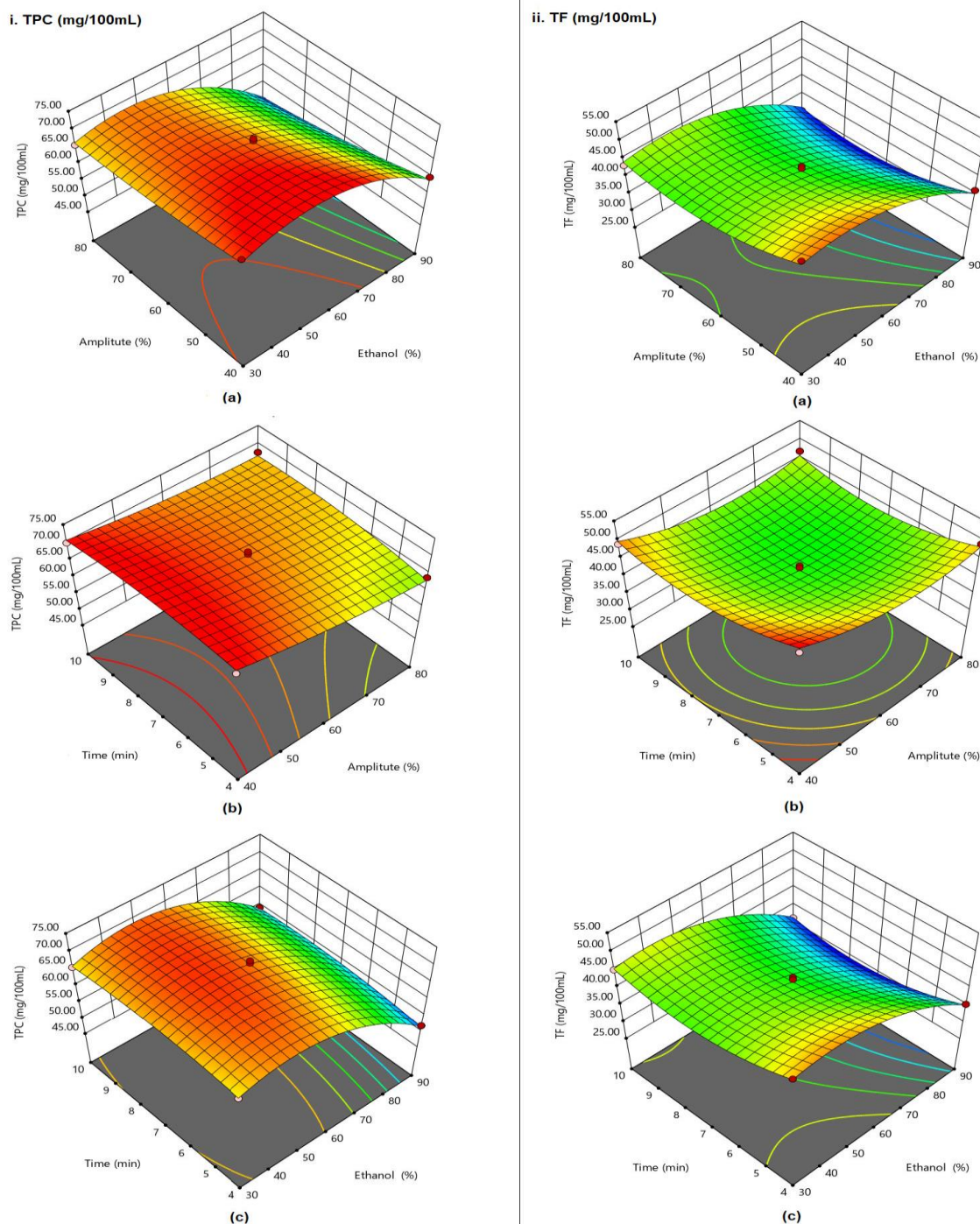

Figure 2. Response surfaces of TPC and TF.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity represents the overall antioxidant activity of extracted bioactive compounds. The ethanol concentration between 50 to 70% has given the highest DPPH activity, as shown in Figure 3 (iv-a). From Figure 3 (iv-b),

the DPPH scavenging activity is increasing with an increase in ultrasound amplitude. The ultrasound treatment time appeared to have very negligible effects on DPPH scavenging activity, as shown in Figure 3 (iv-b and c). The ethanol concentration showed a significant negative impact, whereas ultrasound amplitude and ultrasound treatment time had a significant positive effect on DPPH scavenging activity. The DPPH scavenging activity always may not be directly proportional to total phenol content as scavenging activity is shown by the many compounds such as polyphenols, carotenoids, flavonoids, polysaccharides, etc. [46]. The results of DPPH scavenging activity were found to similar to the outcomes of Altemimi *et al*. (2016), Goula *et al*. (2017), and Altemimi *et al*. (2015).
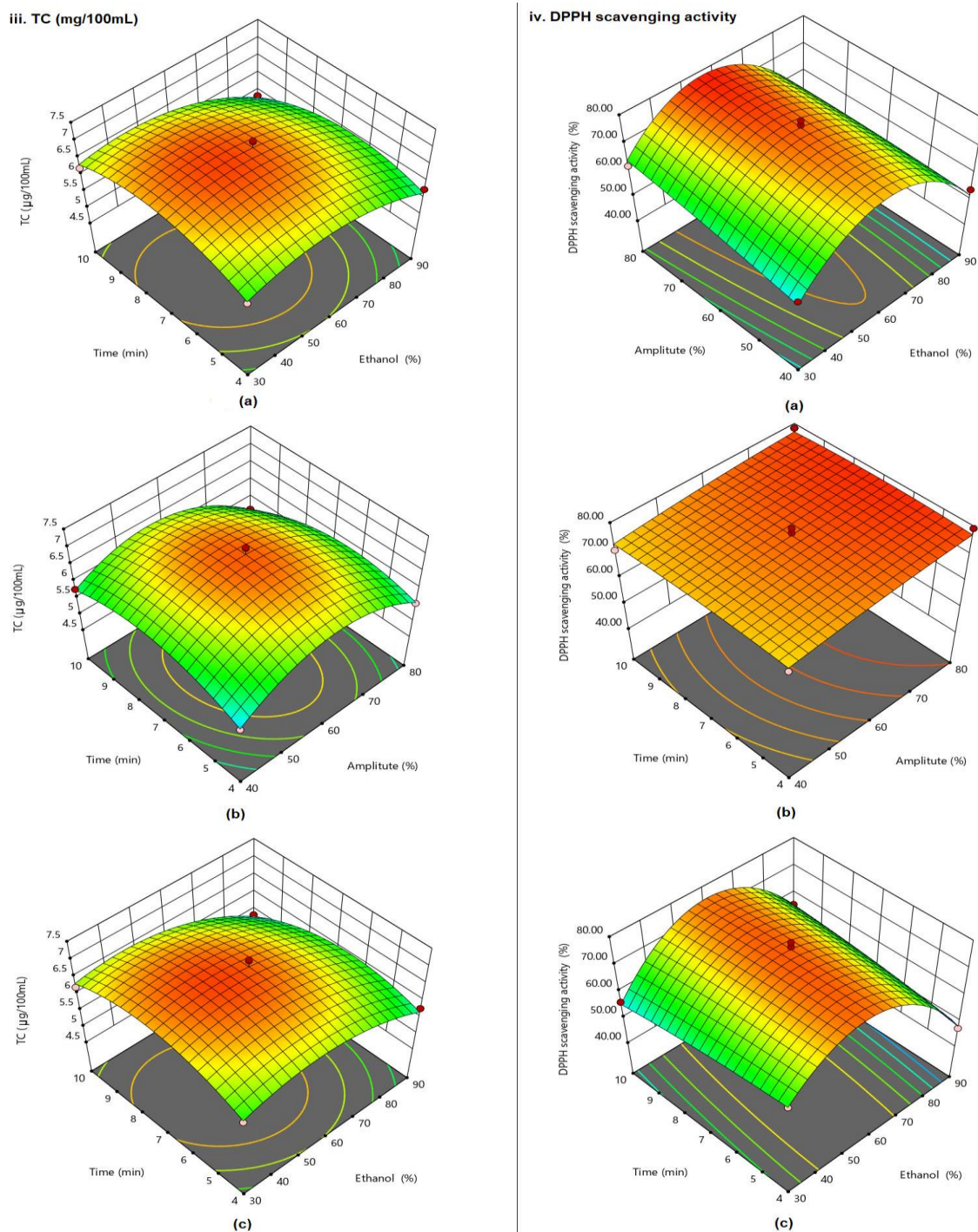

Figure 3. Response surfaces of TC and DPPH scavenging activity.

3.5. Optimization of operational variables.

RSM optimization of variables has been carried out by numerical method. The ethanol concentration of 51.21%, ultrasound amplitude of 51.45%, and ultrasound treatment time of 6.11 minutes were found out to be optimized values of operational variables. Similar kinds of optimal values have been reported by Altemimi *et al*. (2016), Hossain *et al*. (2012), Khan *et al*. (2010), Prasad *et al*. (2011), and Wang *et al*. (2012). The optimized values of TPC, TF, TC, and DPPH scavenging activity were calculated at optimized values of variables and tabulated in Table 4. The desirability bar graph of variables and responses has shown in Figure 4. The overall desirability of the numerical optimization process was found out to be 0.843, which is acceptable [86].

Table 4. RSM-optimized values of variables and responses.

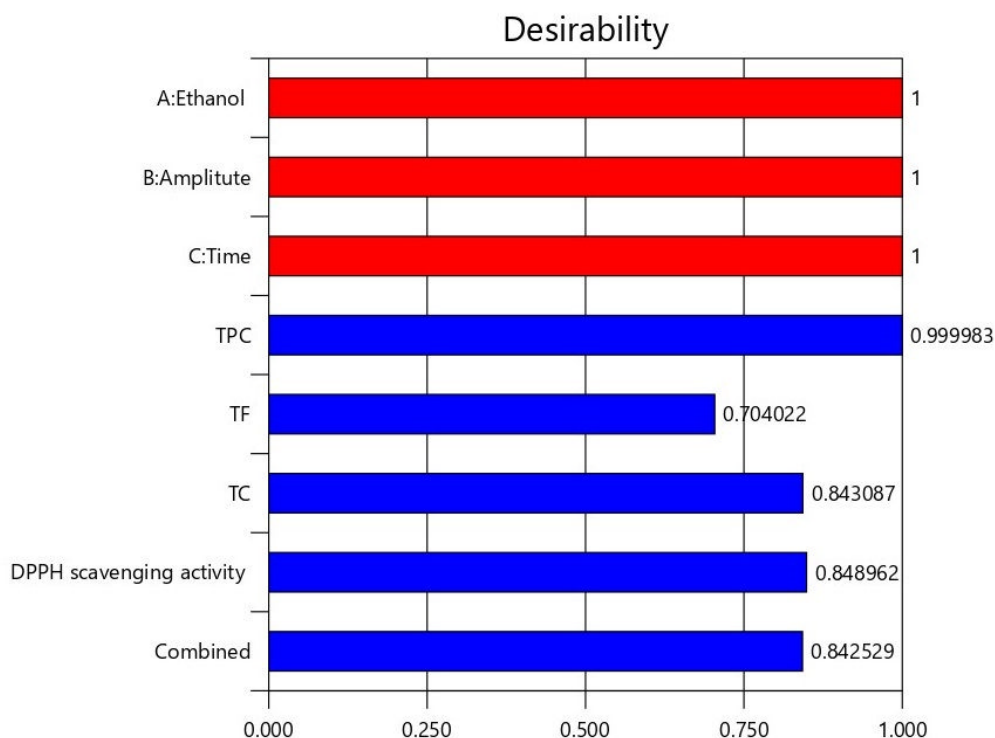| Parameters	RSM optimized values
1. Variables	
Ethanol concentration (%)	51.22
Ultrasound amplitude (%)	51.45
Ultrasound treatment time (minutes)	6.11
2. Responses	
TPC (mg gallic acid equivalent/100mL)	70.79
TF (mg rutin acid equivalent /100mL)	45.85
TC (mg β-carotene equivalent /100mL)	6.75
DPPH scavenging activity (%)	74.40

Figure 4. The desirability of variables and responses for the optimization of the extraction process.

4. Conclusions

The process of bale fruit juice extraction has shown the higher production of bael fruit pulp waste (BFPW) (37.33%), and it was then recycled for the extraction of bioactive compounds to reduce solid food waste and, consequently, the environmental pollution through dumpings. The ultrasound-assisted extraction (UAE) was concluded to have the capability to increase the extraction efficiency of bioactive compounds than conventional solid-liquid

extraction. RSM analysis of Box–Behnken design for the extraction process revealed that the ethanol concentration, ultrasound amplitude, and ultrasound treatment time were statistically significant for the process of extraction of bioactive compounds. Although, ultrasound treatment time had the least influence on the extraction of bioactive compounds than ethanol concentration and ultrasound amplitude. The values of RMSE and MAE were found to be within the acceptable limits and also validated the effectiveness of RSM for the process of ultrasound-assisted extraction of bioactive compounds from BFPW. The RSM analysis showed the ethanol concentration of 51.22%, ultrasound amplitude of 51.45%, and ultrasound treatment time of 6.11 minutes as an optimized value of operational variables for the extraction process of bioactive compounds such as polyphenols, flavonoids, carotenoids, etc. The present study revealed the richness of BFPW in terms of essential compounds that can be recovered efficiently by the ultrasound extraction method. Recovered or extracted compounds may be used either directly in liquid or as a lyophilized powder for its potential use in food and pharmaceutical medicines.

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Conflicts of Interest

The authors declare no conflict of interest.

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